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STRUCTURES OF THE ADULT OVARIES IN DIFFERENT FUNCTIONAL PHASES OF THE PSEUDOSCORPION, *GARYPUS JAPONICUS* BEIER.

I. THE OVARY IN THE RESTING PHASE[1].

By

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Synopsis

Makioka, Toshiki (Shimoda Marine Research Center, University of Tsukuba, Shimoda, Shizuoka 415, Japan): Structures of the adult ovaries in different functional phases of the pseudoscorpion, *Garypus japonicus* Beier. I. The ovary in the resting phase. *Acta Arachnol.*, 28: 71-81 (1979).

Twelve types of cells were distinguished in the adult ovary of the resting phase of *Garypus japonicus* based mainly on their structures and locations. These types of cells were divided into two groups: the slightly differentiated and the well differentiated cell types which compose the ventromedian younger and the dorsolateral older ovarian tissues, respectively. A progressive differentiational gradient from the ventromedian to the dorsolateral portion was presumed.

Since Lubbock's initiative study (1861) on the ovary and embryos of *Chelifer cancroides*, it is known in some species of pseudoscorpions that the adult ovary has two main functions: the formation of the eggs and the secretion of the nutritive fluid for the embryos and larvae. Based on an alternative occurrence of these two functions, four functional phases were distinguished in adult ovary of *Garypus japonicus*: the resting, preparatory, secretory and renovational phases (Makioka, 1976). During these functional phases, the ovary develops well in the periods of egg formation and secretion of the nutritive fluid, and then most of it shrinks into a degenerating cellular mass which is replaced by newly developed ovarian tissues. These transitional changes in the structure of the ovary, however, have never been described in a tissue- or a cellular level.

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In this series of studies, it is planned to describe the cellular construction of adult ovary of *G. japonicus* in each functional phase and to analyse some developmental and functional relations among the ovarian tissues or cells. The present paper deals with the cellular construction of the ovary in the resting phase.

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Materials and Methods

Adult females of *Garypus japonicus* BEIER were collected from the coasts of Shimoda in the Izu Peninsula every month through the years from 1976 to 1978. The ovaries in the resting phase were removed from some of these females immediately after each collection.

The ovaries were fixed with BOUIN's solution, HEIDENHEIN's Susa solution, CARNOY's acetic acid—ethanol solution or 10% neutral buffered formol and prepared as serial paraffin sections 5-10 μm thick or frozen sections 4-8 μm thick. Then, they were stained with MAYER's acid hemalum and eosin, HEIDENHEIN's azan, methyl green and pyronine for DNA and RNA (LISON, 1960) or sudan black B for general lipids (LISON, 1960). These preparations were observed under a light microscope.

Observations

Ovaries in the resting phase are found in almost all females collected during the late autumn, winter and early spring seasons. The ovarian trunk is a transparent tube lying ventromedianly in the abdomen, beneath the S-shaped hindgut, and surrounded loosely by lobes of midgut gland. The anterior part of the ovarian trunk divides into two branches continuing to respective short oviducts which fuse into a median genital chember with a genital pore located between the 2nd and the 3rd ventral plate. The posterior blind end of the ovarian trunk is connected with the ventral surface of the faecal sac or preanal pouch of the hindgut. From the single part of the ovarian trunk, 20-30 tubular egg stalks, each bearing one late vitellogenic oocyte of about 150 μm in diameter, protrude laterally in pairs in regular intervals into the body cavity (Figs. 1, 2-A, 3-A).

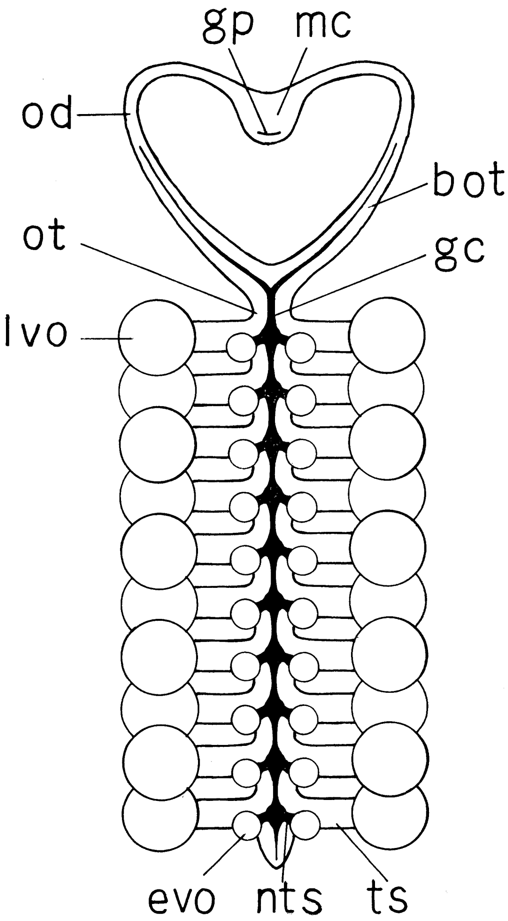
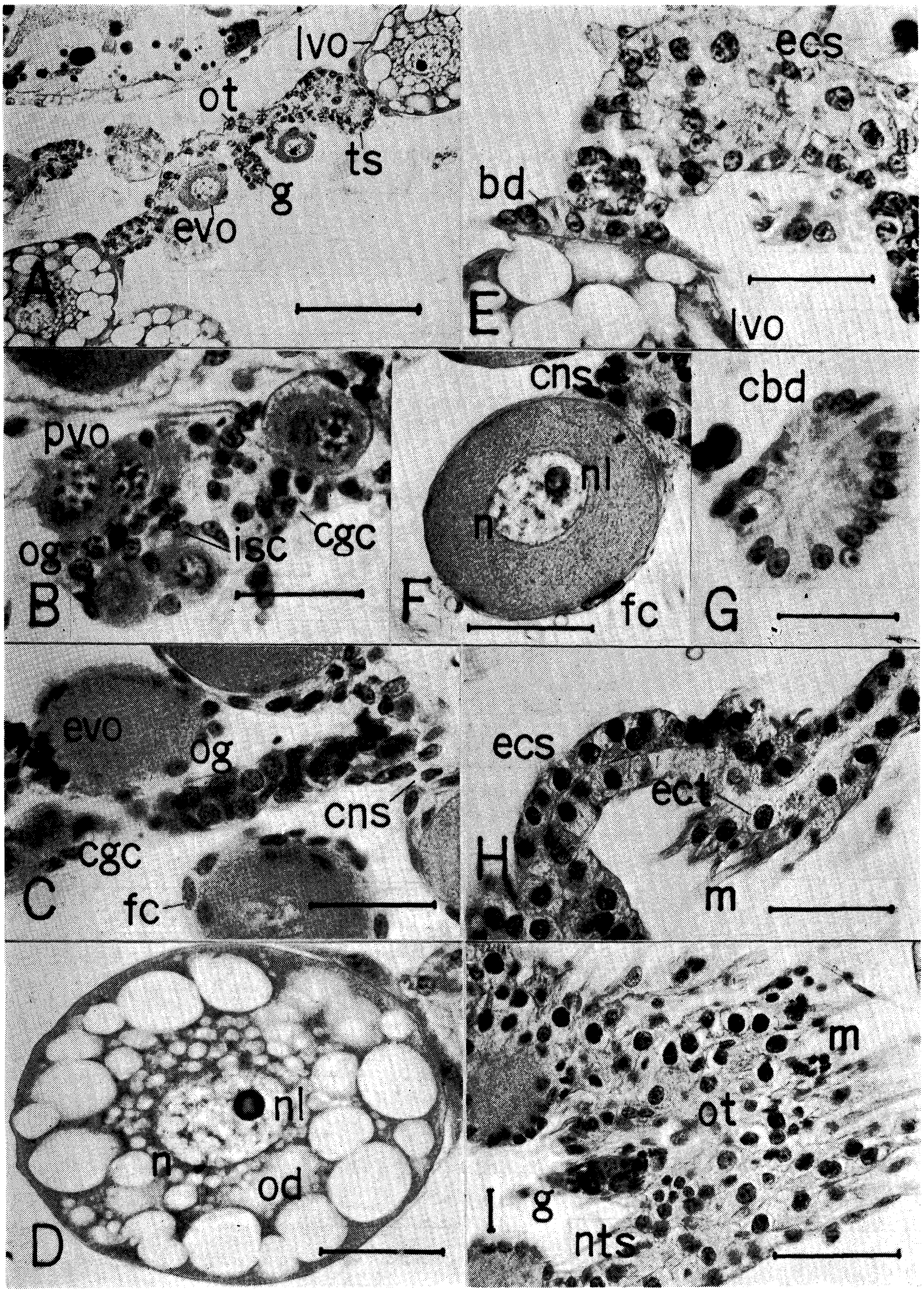


Fig. 1. Diagrammatic representation of the adult ovary in the resting phase (ventral view).
bot: anterior branch of ovarian trunk, evo: early vitellogenic oocyte, gc: germinal cord, gp: genital pore, lvo: late vitellogenic oocyte, mc: median genital chamber, nts: nontubular egg stalk, od: oviduct, ot: ovarian trunk, ts: tubular egg stalk.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On the ventromedian surface of the ovarian trunk, there runs a fine linear cumulus, the germinal cord, extending over about the total length of the ovarian trunk. From both lateral sides of the single part of the germinal cord, 20-30 nontubular egg stalks bearing respective early vitellogenic oocytes of about 80 μm in diameter protrude in the same pattern as the former type of egg stalks. At each protruding point on the ovarian trunk, the egg stalks form a double pair: an outer pair of the formar type and an inner pair of the latter one (Figs. 1, 2-A, 3-A).

The oogonia and the previtellogenic oocytes occur only in 10-15 subspherical germaria arranged moniliformly in the single, part of the germinal cord. The oogonia, about 9 μm in diameter, have several distinct chromatin particles but no distinct nucleoli in their nuclei of about 7 μm in diameter. An oogonial cluster, including 6-10 oogonia, occupies the ventromedian portion of each germarium (Fig. 2-B and C). The dorsolateral portion of each germarium contains 6-12 previtellogenic oocytes, 10-40 μm in diameter, together with several cells of another type, the interstitial cells (Fig. 2-B). The larger the oocytes are, the more dorsolaterally they are located in the germaria. The chromatin in their germinal vesicles shows several typical patterns of early stages of the first reduction division. A distinct nucleolus occurs in the germinal vesicle of each oocyte of more than about 15 μm in diameter. The ooplasm includes neither distinct yolk granules nor oil drops. The interstitial

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Fig. 2. Photomicrographs of ovarian tissues and cells in the resting phase.
A. A transverse section of the ovary. BOUIN—HE. Scale: 100 μm.
B. A transverse section of the germarium. BOUIN—HE. Scale: 33 μm.
C. A horizontal section of the germinal cord. Susa—Azan. Scale: 33 μm.
D. A late vitellogenic oocyte. BOUIN—HE. Scale: 33 μm.
E. A tubular egg stalk. BOUIN—HE. Scale: 33 μm.
F. An early vitellogenic oocyte. BOUIN—HE. Scale: 33 μm.
G. A transverse section of the basal disc. BOUIN—HE. Scale: 33 μm.
H. An ovarian trunk and a tubular egg stalk. BOUIN—HE. Scale.: 50 μm.
I. An obliquely transverse section of the ovarian trunk. BOUIN—HE. Scale: 50 μm.
bd: basal disc, cbd: cell of basal disc, cgc: cell of germinal cord, cns: cell of nontubular egg stalk, ecs: epithelial cell of tubular egg stalk, ect: epithelial cell of ovarian trunk, evo: early vitellogenic oocyte, fc: follicle cell, g: germarium, isc: interstitial cell, lvo: late vitellogenic oocyte, m: muscle fiber, n: nucleus, nl: nucleolus, nts: nontubular egg stalk, od: oil drop, og: oogonium, ot: ovarian trunk, pvo: previtellogenic oocyte, ts: tubular egg stalk. HE: hematoxylin-eosin staining.

cells occur among the previtellogenic oocytes in germaria (Fig. 2-B). Their nuclei, about 6 μm in diameter, include several clusters of chromatin but no distinct nucleoli.

Both the germinal cord and the nontubular egg stalks bearing early vitellogenic oocytes consist of cells of the same shape which are similar in size and in structure to the interstitial cells of the germaria (Fig. 2-B, C and F). Each early vitellogenic oocyte has a spherical nucleolus consisting of a peripheral basophilic and a central acidophilic component in the germinal vesicle of the diffusing stage (Fig. 2-F). The ooplasm includes rarely a few small oil drops but no distinct proteid yolk granules. Each early vitellogenic oocyte is surrounded by a follicle layer which consists of several flat follicle cells (Fig. 2-C and F). The nucleus of each follicle cell resembles apparently those of adjacent cells of the nontubular egg stalk, but it is remarkably flattened (about 7 μm in diameter and about 3 μm in height) and has often one or two distinct nucleoli.

The walls of the ovarian trunk and the tubular egg stalks bearing the late vitellogenic oocytes have a common columnar epithelium surrounding the common lumen (Fig. 2-H). Each epithelial cell, about 10 μm in diameter and about 20 μm in height, has several clusters of chromatin and one or two distinct nucleoli in the nucleus of about 8 μm in diameter. A rudimentary muscle layer consisting of some striated muscle fibers arranged longitudinally surrounds the epithelium of the ovarian trunk (Fig. 2-H and I). The epithelia of the tubular egg stalks are not surrounded by the muscle layer (Fig. 2-E and H). At the distal end of each tubular egg stalk, there is a dish-like tissue on which the late vitellogenic oocyte is held (Fig. 2-E). This discoidal tissue seems to keep the oocyte from migrating through the lumen of the egg stalk. According to its shape and position, this tissue was termed "basal disc" of the late vitellogenic oocyte in this paper. The basal disc consists of some conical cells, about 8 μm in maximal diameter and about 30 μm in height, arranged in a rosette pattern (Fig. 2-G). These cells have more acidophilic cytoplasm and more diluted chromatin than those of adjacent epithelial cells of the egg stalk. One or two distinct nucleoli are found in their respective nuclei of about 7 μm in diameter. Each late vitellogenic oocyte has a large germinal vesicle of the same construction as that of an early vitellogenic oocyte. Its ooplasm is filled with a number of large oil drops. A thin egg membrane surrounds the oocyte, but no follicle cells are found around the oocyte (Fig. 2-D).

Discussion

The ovarian cells of adult *Garypus japonicus* are distinguished into twelve types based mainly on their structures and locations. Such a fine division of cell types seems necessary to describe some differences in ovarian structures in different functional phases and to analyze the origins and fates of the ovarian tissues and cells.

1. *Oogonia*

SAREEN (1965) studied their structure and histochemical nature for the first time in *Diplotemnus insolitus* but he did not describe their locality in the ovary. WEYGOLDT (1969) found them in the germ cell layer of the ventral wall of the ovarian tube in *Pselaphochernes scorpioides*. In *G. japonicus*, they occur in the ventromedian portion of the germaria.

2. *Previtellogenic oocytes*

SAREEN (1965) studied their structure and histochemical nature. WEYCOLDT (1969) suggested first that they occurred in the germ cell layer accompanied by neither the follicle epithelia nor the egg stalks. In *G. japonicus*, they occur in the dorsolateral portion of the germaria together with the interstitial cells.

3. *Early vitellogenic oocytes*

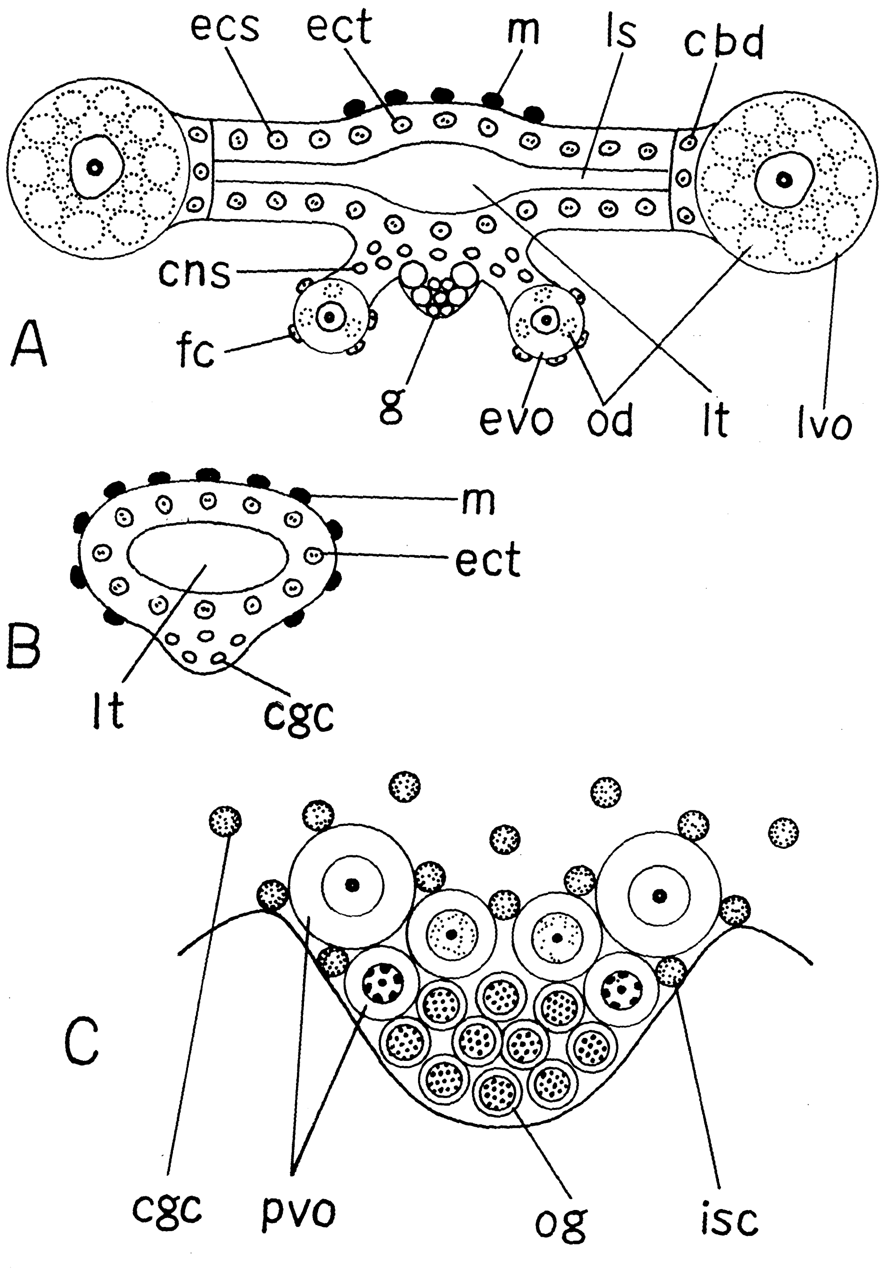
In the pseudoscorpions, the vitellogenic oocytes produce scarcely any proteid yolk, but a great deal of fatty yolk or oil (SAREEN, 1965; MAKIOKA, 1968; BOISSIN et MANIER, 1969-1970; BOISSIN, 1970). WEYGOLDT (1969) showed various sizes of vitellogenic oocytes with respective follicle layers and egg stalks in one ovary which seems to be in the resting or the preparatory phase. In *G. japonicus*, however, there are only two regular sizes of vitellogenic oocytes in one ovary during the resting and the preparatory phase: the early and the late vitellogenic oocytes (MAKIOKA, 1976). The early vitellogenic oocytes are accompanied by respective follicle layers and nontubular egg stalks. A few small oil drops are found rarely in their ooplasm.

4. *Late vitellogenic oocytes*

Their ooplasm is filled with a number of large oil drops. They are accompanied by respective tubular egg stalks and basal discs but no follicle layers.

5. *Cells of the germinal cord*

MAKIOKA (1976) described them preliminarily as "interstitial cells" together with the interstitial cells of the present paper. These two types of cells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resemble each other closely in shape and in size, but not in location and possible fate.

6. *Cells of the nontubular egg stalks*

VACHON (1938) described first in *Chelifer cancroides* that the egg stalks bearing young oocytes had a nontubular structure. In *G. japonicus* also, these cells are not in epithelial arrangement. They resemble the cells of the germinal cord and the interstitial cells in their slightly differentiated structure. WEYGOLDT (1969) regarded the egg stalk as a part of the follicle layer elongating with growth of the vitellogenic oocyte. In *G. japonicus*, however, the cells of the nontubular egg stalks are different from the follicle cells in structure, in manner of arrangement and in possible fate.

7. *Interstitial cells*

They occur in the dorsolateral portion of the germaria, among the previtellogenic oocytes. They are most probably prefollicle cells.

8. *Follicle cells*

VACHON (1938) described first that these cells surrounded the young oocytes. In *G. japonicus*, they are more specialized cells than the adjacent cells of types 5, 6 and 7. They are arranged in a layer of flat epithelium. One or two distinct nucleoli are often found in their nuclei.

9. *Cells of the basal disc*

VACHON (1938) described them first as the degenerating follicle cells. In *G. japonicus*, they compose a well defined tissue, the basal disc, possibly with some inherent functions. They are different from adjacent epithelial cells of the tubular egg stalk in structure, in stainability and in manner of arrangement.

10. *Epithelial cells of the tubular egg stalks*

They construct the wall of the tubular egg stalk. VACHON (1938) and MAKIOKA (1968) described that they did not take part in secretion of the

Fig. 3. Diagrammatic representation of transverse sections of the ovarian trunk.

A. A section at the protruding point of egg stalks.

B. A section between the protruding points of egg stalks.

C. A section of a germarium.

cbd: cell of basal disc, cgc: cell of germinal cord, cns: cell of nontubular egg stalk, ecs: epithelial cell of tubular egg stalk, ect: epithelial cell of ovarian trunk, evo: early vitellogenic oocyte, fc: follicle cell, g: germarium, isc: interstitial cell, ls: lumen of tubular egg stalk, lt: lumen of ovarian trunk, lvo: late vitellogenic oocyte, m: muscle cell, og: oogonium, pvo: previtellogenic oocyte.

nutritive fluid. In *G. japonicus*, however, they secrete the nutritive fluid in the secretory phase together with the epithelial cells of the ovarian trunk (MAKIOKA, in preparation for publication).

11. *Epithelial cells of the ovarian trunk*

They construct the inner wall of the ovarian trunk. VACHON (1938) described first that they secreted the nutritive fluid. In *G. japonicus*, they resemble the epithelial cells of the tubular egg stalks in structure, in manner of arrangement and in possible fate; the only difference is their location.

12. *Muscle cells*

They construct the outer wall of the ovarian trunk forming a rudimentary longitudinal muscle layer. MAKIOKA (1968) described that there was no muscle layer around the ovarian trunk, because he mistook the epithelium of the tubular egg stalk for that of the ovarian trunk in the secretory phase of *G. japonicus*.

These twelve cell types are divided into two groups: the slightly differentiated and the well differentiated cell types. Cell types of the former group, types 1, 2, 5, 6 and 7, are localized in the ventromedian minor portion of the ovary (Fig. 3-A. B and C). Neither distinct nucleoli nor metaplasmic or deutoplasmic products are found in these cell types, excepting the previtellogenic oocytes of more than about 15 μm in diameter which have a distinct nucleolus in their respective nuclei. On the other hand, cell types of the latter group, types 3, 4, 8, 9, 10, 11 and 12, occupy the dorsolateral major portion of the ovary, forming some specialized tissues: the epithelia of the tubular egg stalks and of the ovarian trunk, the follicle layer and the muscle layer (Fig. 3-A and B). These types of cells have distinct nucleoli and some metaplasmic or deutoplasmic products such as oil drops, muscle fibrils, egg membranes, etc. As the only exception, the follicle cells have no distinct nucleoli in some cases.

It is presumed, based on the above mentioned structure, that the ovary has a progressive differentiational gradient from the ventromedian to the dorsolateral portion (Fig. 3-A). Accordingly, it is expected that the ventral younger tissues resting on their ways of differentiation subsequently become the next dorsal older tissues, replacing the degenerating parts of the ovary. However, the developmental relations between these ovarian tissues should be discussed after some detailed observations on the ovarian structures in other functional phases have finished.

摘　　要

牧岡俊樹（筑波大学下田臨海実験センター，〒415 静岡県下田市5-10-1）：異なる機能期におけるイソカニムシ成体卵巣の構造．Ⅰ．休止期の卵巣．

1．イソカニムシ *Garypus iaponicus* 成体卵巣の4つの機能期のうち，休止期の卵巣の細胞および組織構成が観察された。

2．休止期の卵巣を構成する12の cell types が区別された。これらは，分化の程度の低い5つの cell types と，よく分化した7つの cell types の2群から成る。

3．分化の程度の低い cell types は，卵巣の腹側中央部を占め，形成細胞巣を含む生殖紐や非管状卵柄などの若い組織を作る。

4．よく分化した cell types は，卵巣背側の主要部において，卵巣幹上皮，筋層，管状卵柄の上皮，基底盤，沪胞上皮などのよく分化した組織を作る。

5．これらの組織の配置にもとづいて，卵巣の腹側中央部から背側方にむかう分化の勾配の存在が推定された。

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